FACTORS ASSOCIATED WITH SEVERE CLINICAL MANIFESTATION OF DENGUE AMONG ADULTS IN THAILAND

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Abstract. A retrospective study was conducted by reviewing medical records of 323 adult patients hospitalized with dengue infection at the Hospital for Tropical Diseases, Bangkok, Thailand between 2006 and 2010 in order to determine factors associated with severe clinical manifestations of dengue. Of 323 patients, 95 (29.4%) had dengue fever (DF) and 228 (70.6%) had DHF, which were categorized as grade I (67 patients, 29.4%), grade II (62 patients, 27.2%), grade III (95 patients, 41.7%) and grade IV (4 patients, 1.8%) following 1997 WHO definitions. Using the revised 2009 WHO definitions, 233 patients (72.1%) had non-severe dengue infection and 90 patients (27.9%) had severe dengue infection. Of the 233 patients with non-severe dengue infection, 193 (82.8%) were classified as having non-severe dengue infection with warning signs and 40 (17.2%) were classified as having non-severe dengue infection without warning signs. Using stepwise multivariate logistic regression analysis, having a hematocrit >2% above the reference range [odds ratio (OR) 3.235; 95% confidence interval (CI) 1.807-5.793] or having an alanine aminotransferase level >120 IU/l (OR 1.896; 95% CI 1.018-3.531) were associated with having DHF grades II-IV, whereas female gender (OR 2.042; 95% CI 1.143-3.648) or having a mean arterial pressure <80 mmHg (OR 2.275; 95% CI 1.302-3.975) were associated with severe dengue. These findings may help clinicians to determine patients at risk for severe manifestations of dengue infection, which could lead to proper management of these cases.

Keywords: dengue hemorrhagic fever, severe dengue, associated factors, Thailand

INTRODUCTION

Dengue infection is a mosquito-borne viral disease with four serotypes and is re-emerging throughout the world, especially in tropical countries (WHO, 1997). Dengue infection mainly affects children, but the numbers of adult cases of dengue infection have increased dramatically in recent decades (Wichmann *et al*, 2004). Laboratory diagnosis of dengue infection is based on detection of viral components in the serum. This includes testing for viral nucleic acid by reverse-transcriptase polymerase chain reaction (RT-PCR), virus-expressed soluble non-structural protein 1 (NS1) antigen, or the detection

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of specific dengue immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies. These tests are expensive, time consuming and may not be available in many hospitals, especially in developing countries (Schwartz *et al*, 2000; Clark *et al*, 2005). Therefore, the diagnosis of dengue infection usually relies on clinical manifestations (Potts and Rothman, 2008).

The World Health Organization (WHO) has developed guidelines for the diagnosis of dengue infection based on clinical and laboratory findings. The 1997 WHO definition differentiates dengue into three categories: undifferentiated fever, dengue fever (DF) and dengue hemorrhagic fever (DHF) (WHO, 1997). However, there are several case reports of unusual manifestations of dengue infection including hepatitis, encephalitis, and multi-organ failure which could not be classified using the 1997 WHO definition (Bandyopadhyay *et al*, 2006). Furthermore, this definition makes it difficult to predict disease severity and clinical outcomes (Balmaseda *et al*, 2005).

In 2009, the WHO adopted and published a revised case definition for the diagnosis of dengue infection. For non-severe dengue, warning signs are taken into account in order to predict severe dengue and provide proper case management (WHO, 2009). Currently, the 1997 WHO definition continues to be widely used but the revised criteria have the potential to be applicable in clinical practice and accepted by health care providers (Basuki *et al*, 2010; Barniol *et al*, 2011). However, there are limited data on factors associated with severe clinical manifestations of dengue infection. Therefore, we conducted a retrospective study reviewing the medical records of adult dengue infection patients admitted to the Hospital for Tropical Diseases, Bangkok, Thailand in order to determine factors associated with the severe clinical manifestations of dengue infection using the 1997 WHO definition and the revised 2009 WHO definition.

MATERIALS AND METHODS

Study design

This retrospective study was conducted at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand and was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The medical records of hospitalized patients diagnosed as having dengue infection at discharge between 2006 and 2010 were retrieved using the ICD-10 code for dengue infection; those which fulfilled the study criteria were reviewed. The study criteria were: adult patients aged ≥15 years with clinical criteria for dengue infection and a laboratory diagnosis of dengue infection, including a positive dengue NS1 antigen rapid test, a positive RT-PCR, or a positive dengue IgM rapid test from a single serum sample during hospitalization (Lanciotti *et al*, 1992; Reynes *et al*, 2003). Patients who met clinical criteria for having dengue infection and who had a positive dengue NS1 antigen test or a positive dengue IgM rapid test were defined as probable dengue patients and patients who had clinical criteria for dengue and had a positive RT-PCR test were defined as confirmed dengue patients. Patients with mixed infections were excluded from this study. Patient data, including demographics, clinical manifestations, laboratory findings, management and outcomes were extracted from the hospital records and discharge summaries using a predefined case record form were recorded.

The severity of dengue infection was evaluated using the 1997 WHO definition and the revised 2009 WHO definition. The most severe grading for the patient during hospitalization was used as the final disease severity.

Case definition of dengue

According to the 1997 WHO definition (WHO, 1997), DF is defined as an acute fever with ≥2 of the following criteria: 1) headache, 2) retro-orbital pain, 3) myalgia or arthralgia, 4) rash, 5) a positive tourniquet test defined as the presence of ≥20 petechiae per 1 square inch, and 6) leukopenia defined as a white blood cell count (WBC) $<4.0 \times 10^3$ cells/ l. DHF was classified if the following four criteria were met: 1) having an acute fever or history of fever lasting 2-7 days, 2) having thrombocytopenia defined as a platelet count $<100 \times 10^3$ cells/ l; 3) having spontaneous bleeding into the skin, or from the nose, gums, gastrointestinal tract or vagina, and 4) having plasma leakage defined as a rise in hematocrit (Hct) ≥20% above reference range for a healthy Thai adult adjusted for gender or having evidence of clinical fluid accumulation as manifested by a pleural effusion or ascites. Severity of DHF was further classified into four grades: grade I DHF defined as DHF patient having spontaneous bleeding into the skin manifested by petechiae, ecchymosis or a positive tourniquet test; grade II DHF defined as a DHF patient having spontaneous bleeding from the nose, gums, gastrointestinal tract or vagina; a grade III DHF was defined as a DHF patient having hypotension manifested by a pulse pressure <20 mmHg or a systolic blood pressure <90 mmHg; and grade IV DHF was defined as a DHF patient having shock, manifested by hypotension and evidence of poor tissue perfusion such as decreased urine output (<0.5 ml/kg/hr).

According to the revised 2009 WHO definition (WHO, 2009), dengue patients were classified into non-severe dengue and severe dengue based on clinical and laboratory criteria. Patients with non-severe dengue were sub-categorized into two groups depending on the presence or absence of warning signs. Non-severe dengue without warning signs was defined as having acute fever with ≥2 of the following criteria: 1) nausea, 2) rash, 3) myalgia or arthralgia, 4) a positive tourniquet test, and 5) leukopenia. The warning signs included 1) abdominal pain or tenderness, 2) severe vomiting defined as vomiting with signs of dehydration on physical examination, 3) plasma leakage, and 4) spontaneous bleeding from the nose or gums. Severe dengue was classified as those having 1) severe plasma leakage, defined as having plasma leakage with shock or respiratory distress as manifested by a respiratory rate >24 breaths/minute and/or required oxygen therapy; 2) having severe clinical bleeding defined as spontaneous bleeding from the gastrointestinal tract and/or vagina and/or requiring a blood transfusion and/or receiving other acute management for controlling active bleeding such as nasal packing or a dental splint; and 3) having severe organ involvement defined as an aspartate aminotransferase (AST) level ≥1,000 IU/l and/or an alanine aminotransferase (ALT) level ≥1,000 IU/l and/or a serum creatinine >1.2 mg/dl.

DHF grades II, III and IV categorized by the 1997 WHO definition and severe dengue categorized by the revised 2009 WHO definition were defined as severe clinical manifestations of dengue in our study. In our hospital, the decision to admit a dengue patient is up to the treating physician. The indications for admission of the dengue patient at our hospital are

not always well documented, but typically included patients with dehydration, severe vomiting, abdominal pain or tenderness, clinical bleeding and thrombocytopenia with concurrent fever.

Immunochromatographic detection of dengue NS1 antigen

The dengue NS1 antigen was detected using a commercially available rapid test (SD Bioline, St. Ingbert, Germany) according to the manufacturer's instructions. This rapid test is an *in vitro* immunochromatographic one step assay for the determination of dengue virus NS1 antigen.

Reverse-transcriptase polymerase chain reaction (RT-PCR)

Dengue viral RNA in a patient's serum is extracted with the Boom method as previously described (Boom *et al*, 1990). The extraction is then subjected to RT-PCR using dengue specific anti-sense D2 primer and the products are used as a template for nested-PCR reaction using D1, TS1, TS2, TS3 and TS4 primers as described by Lanciotti *et al* (1992).

Serology

Serum was tested for anti-dengue viral IgM antibodies using a rapid immunochromatographic assay (SD Bioline, St. Ingbert, Germany) according to the manufacturer's instructions.

Sample size calculation

Several factors associated with severe clinical manifestations of dengue infection were evaluated. The estimation of the number of subjects included in this study was important. We estimated each associated factor's corresponding number and considered the highest estimated number to allow for appropriate analysis of the association between possible associated factors and severe clinical manifestations of dengue.

The sample size was estimated using the Power and Sample Size Program, version 3.0, 2009 (Dupont WD, Creative Commons Attribution-Non Commercial-NoDerivs, 3.0, United States). The occurrence of severe clinical manifestations among hospitalized patients with dengue was expected to be 50% in our study. Therefore, a study was planned to have the outcomes with the ratio of non-severe clinical manifestations to severe clinical manifestations of 1:1. The probability of exposure to an associated factors among patients with non-severe clinical manifestations was estimated to be 0.25. If the true odds ratio for a severe clinical manifestation in an exposed subject relative to an unexposed subject is 2.5, we needed to study at least 123 dengue infected patients with severe clinical manifestations and 123 dengue infected patients with non-severe clinical manifestations to be able to reject the null hypothesis that this odds ratio equals 1 with 90% power and a Type I error probability associated with this test if the null hypothesis is 0.05. Therefore, the required number of cases was at least 246 dengue infected patients.

Statistical analysis

Data were analyzed using the statistical package SPSS for Windows 18.0 (SPSS, Chicago, IL). Numerical variables were tested for normality using the Kolmogorov-Smirnov test. Variables with non-normal distribution were summarized as median and inter-quartile range (IQR) and compared with the Mann–Whitney *U* test for 2 group comparison. Categorical variables were expressed as frequencies and percentages then analyzed with the chi-square test or the Fisher's exact test where appropriate. Univariate analysis was performed to determine factors associated with severe clinical manifestations of

dengue infection. Any variable with $p \leq 0.2$ on univariate logistic regression analysis was considered statistically significant and then further analyzed in the stepwise multivariate logistic regression analysis using the backward selection method for determining independent associated factors for severe clinical manifestations of dengue infection. All tests of significance were 2-sided, with a $p<0.05$ indicating statistical significance.

RESULTS

A total of 486 medical records of adult hospitalized patients with a diagnosis of dengue infection using the ICD-10 code for dengue were retrieved; 163 of these were excluded due to the lack of laboratory confirmation of dengue infection (158 cases) or having mixed-infection (5 cases). Thus, 323 medical records fulfilling the study criteria were reviewed. Of the 323 medical records reviewed, only 4 were confirmed to be dengue infection by RT-PCR and the rest were cases of probable dengue infection diagnosed on the basis of a positive test result with a dengue IgM rapid test in 219 out of 232 (94.4%) or by detecting dengue NS1 antigen in 118 out of 171 cases (69.0%). The median (IQR) time to positivity with the RT-PCR detecting dengue infection was 4.0 (4.0-5.5) days, with the dengue IgM rapid test it was 6.0 (5.0-6.0) days and with the dengue NS1 antigen test, it was 4.0 (3.0-4.0) days after the onset of fever.

Baseline characteristics, laboratory data and hospital course of dengue patients

Of 323 patients with dengue infection, 164 (50.8%) were male with median (IQR) age of 23.0 (19.0-34.0) years. Previous medical illness was reported in 69 patients (21.4%) including asthma or allergic rhinitis (21, 30.4%), diabetes mellitus (16, 23.2%), hyperlipidemia (16, 23.2%), hypertension (13, 18.8%), thalassemia (6, 8.7%), peptic ulcer or gastro-esophageal reflux disease (5, 7.2%), heart disease (3, 4.3%) and others (16, 23.2%). The median (IQR) duration of fever prior to admission was 4.0 (3.0-5.0) days. All patients presented with fever, followed by nausea (227, 70.3%), headache (226, 70.0%), myalgia or arthralgia (179, 55.4%) and vomiting (175, 54.2%). Other symptoms included abdominal pain or tenderness (99, 30.7%), diarrhea (97, 30.0%), petechiae (76, 23.5%), rash (63, 19.5%) and retro-orbital pain (15, 4.6%). The median (IQR) temperature on admission was 37.9 (37.2-38.5)°C. A tourniquet test was not routinely performed because of patient discomfort but the test was performed and the results documented in 26 patients (8%), of which 21 (80.7%) had positive results. Other findings on physical examinations were within normal limits.

Abnormal laboratory findings on admission were observed. The median (IQR) of hemoglobin (Hb) was 14.1 (12.8-15.2) g/dl; Hct, 42.1 (38.7-45.3) %; WBC, 2.9 (2.1-4.2) x 10^3cells/ l; and platelet count, 73.0 (44.0-97.0) x 10^3cells/ l. Liver function tests were performed: AST [median (IQR), 114.0 (63.0-186.0) IU/l, $n = 235$]; and ALT [71.0 (42.0-121.0) IU/l, $n = 236$]. Renal function tests were also performed: blood urea nitrogen [median (IQR), 10.0 (8.0-13.0) mg/dl, $n = 185$] and serum creatinine [0.8 (0.7-1.0) mg/dl, $n = 200$].

During hospitalization, all patients received paracetamol but 8 patients (2.5%) received non-steroidal anti-inflammatory drugs to relieve fever and/or body aches. Other drugs prescribed included proton pump inhibitors (94, 29.1%) and histamine H_2 antagonists or antacid (18, 5.6%) for prevention and/or treatment of gastroin-

testinal bleeding. The majority of patients had evidence of plasma leakage (161, 49.8%); of which 99 (61.5%) had hypotension or shock and 6 (3.7%) patients had respiratory distress. Other complications included spontaneous bleeding from the nose or gums (69, 21.4%) and/or gastrointestinal tract or vagina (40, 12.4%); acute kidney injury (12, 3.7%); and/or a AST or ALT ≥1,000 IU/l (6, 1.9%). Only 11 patients (3.4%) received platelet transfusion. The median (IQR) duration of hospitalization was 3.0 (2.0-4.0) days and none of the patients died (Table 1).

Findings by classification group using the 1997 and 2009 WHO definitions

Of the 323 study subjects, 95 (29.4%) had dengue fever (DF) and 228 (70.6%) had dengue hemorrhagic fever (DHF) according to 1997 WHO definitions. Of the 228 patients with DHF, 67 (29.4%) had grade I DHF, 62 (27.2%) had grad 2 DHF, 95 (41.7%) had grade 3 DHF and 4 (1.8%) had grade 4 DHF. Using revised 2009 WHO definitions, 233 subjects (72.1%) had non-severe dengue infection and 90 (27.9%) had severe dengue infection. Of the patients with severe dengue infection, 72 (80%) had severe bleeding, 12 (13.3%) had renal insufficiency, 6 (6.7%) had an AST or ALT ≥1,000 IU/l, 6 (6.7%) had respiratory distress and 4 (4.4%) had severe plasma leakage with shock. Of the 233 patients with non-severe dengue, 193 (82.8%) had non-severe dengue infection with warning signs and 40 (17.2%) had non-severe dengue without warning signs.

The signs and symptoms of the dengue infected patients classified using the 1997 WHO definitions are shown in Table 1. The baseline characteristics of patients with DHF grades II-IV and those with DF and DHF grade I were similar. The majority of laboratory findings in the two groups of patients are similar except hemoglobin level [median (IQR), 14.8 (13.7-16.0) g/dl versus 13.5 (12.4-14.7) g/dl, $p<0.001$], hematocrit [44.2 (40.9-46.8) % versus 40.1 (36.9-43.2) %, $p<0.001$], AST level [123.0 (71.0-228.0) IU/l versus 106.5 (56.0-160.5) IU/l, $p=0.024$] and ALT level [84.5 (44.5-139.5) IU/l versus 62.0 (40.3-101.0) IU/l, $p=0.023$], which were significantly higher in patients with DHF grades II-IV. When these parameters were classified into categories based on reference range, a significantly greater proportion of patients with DHF grades II-IV had a Hct >2% above reference range [130 (80.7%) versus 91 (56.2%), $p<0.001$] and an ALT level >120 IU/l [39 (32.5%) versus 22 (19.0%), $p=0.026$]. However, the median platelet count [median (IQR), 65.0 (37.0-95.0) x 10^3cells/ l versus 80.0 (53.0-105.5) x 10^3cells/ l, $p=0.002$] was significantly lower among patients with DHF grades II-IV.

Regarding the revised 2009 WHO definitions, patients with severe dengue and those with non-severe dengue with warning signs were compared (Table 2). The majority of clinical and laboratory parameters in the patients with severe dengue and non-severe dengue with warning signs were similar except for female gender [60 (66.7%) versus 78 (40.4%), $p<0.001$], a mean arterial pressure (MAP) <80 mmHg [38 (42.2%) versus 40 (20.7%), $p<0.001$], and a Hct <2% below the reference range [72 (80.0%) versus 122 (63.2%), $p=0.007$], which were observed in a higher proportion of patients with severe dengue. The MAP [median (IQR), 80.2 (73.3-86.7) mmHg versus 83.3 (80.0-93.3) mmHg, $p<0.001$]; Hb [13.5 (12.2-15.0) g/dl versus 14.6 (13.3-15.5), $p<0.001$] and Hct [40.7 (36.8-44.4) % versus 43.5 (40.0-45.9) %, $p<0.001$] on admission were significantly lower in patients with severe dengue.

Table 1
Baseline characteristics and laboratory findings of study subjects with dengue infection classified according to the 1997 WHO definitions.

Characteristics	DHF grades II-IV		Dengue fever and DHF grade I		*p*-value
	n	Median (IQR[a])	*n*	Median (IQR[a])	
Baseline characteristics					
Age (years)	161	24.0 (20.0-34.0)	162	23.0 (19.0-34.0)	0.488
Gender: male, no. (%)	161	89 (55.3)	162	75 (46.3)	0.133
Other medical illnesses, no. (%)	161	36 (22.4)	162	33 (20.4)	0.764
Fever (days)	161	4.0 (3.0-5.0)	162	4.0 (3.0-5.0)	0.293
Headache, no. (%)	161	114 (70.8)	162	113 (69.8)	0.932
Vomiting, no. (%)	161	89 (55.3)	162	86 (53.1)	0.777
Myalgia or arthralgia, no. (%)	161	86 (53.4)	162	92 (56.8)	0.619
Abdominal pain, no. (%)	161	52 (32.3)	162	47 (29.0)	0.603
Verginal bleeding, no. (%)	161	18 (11.2)	162	25 (15.4)	0.337
Rash, no. (%)	161	14 (8.7)	162	16 (9.9)	0.863
Retro-orbital pain, no. (%)	161	7 (4.3)	162	8 (4.9)	1.000
Physical findings					
Temperature (°C)	161	37.8 (37.3-38.5)	162	38.0 (37.2-38.5)	0.578
Mean arterial pressure (mmHg)	161	83.3 (76.7-93.3)	162	83.3 (76.7-90.0)	0.791
Petechiae, no. (%)	161	37 (23.0)	162	39 (24.1)	0.920
Laboratory findings					
Hemoglobin (g/dl)	161	14.8 (13.7-16.0)	162	13.5 (12.4-14.7)	<0.001
Hematocrit (%)	161	44.2 (40.9-46.8)	162	40.1 (36.9-43.2)	<0.001
White cell counts (x10^3/ l)	161	3.0 (2.1-4.5)	162	2.8 (2.1-3.9)	0.238
Lymphocytes (%)	161	29.0 (21.0-41.0)	162	31.3 (21.0-43.9)	0.439
Atypical lymphocytes (%)	161	4.0 (0-10.0)	161	3.0 (0-10.0)	0.279
Platelet count (x10^3/ l)	161	65.0 (37.0-95.0)	162	80.0 (53.0-105.5)	0.002
AST[b] (IU/l)	119	123.0 (71.0-228.0)	116	106.5 (56.0-160.5)	0.024
ALT[c] (IU/l)	120	84.5 (44.5-139.5)	116	62.0 (40.3-101.0)	0.023
Albumin (g/dl)	63	4.2 (3.9-4.4)	59	4.1 (3.9-4.3)	0.643
Serum creatinine (mg/dl)	102	0.9 (0.7-1.0)	98	0.8 (0.7-1.0)	0.271
Categorical data					
Hematocrit >2%, no (%)	161	130 (80.7)	162	91 (56.2)	<0.001
ALT[c] >120 IU/l, no (%)	120	39 (32.5)	116	22 (19.0)	0.026

[a]IQR, interquartile range; [b]AST, aspartate aminotransferase; [c]ALT, alanine aminotransferase

Univariate and multivariate analyses

The clinical and laboratory parameters on admission were analyzed for independent factors associated with severe clinical manifestations of dengue (Table 3). Univariate analysis of patients with DHF grades II-IV following the 1997 WHO definitions for severe clinical manifestations showed a Hct >2% above the reference range [OR (95% CI), 3.272 (1.985-5.393), $p<0.001$] and an ALT >120 IU/l [2.057 (1.128-3.753), $p=0.019$] were factors significantly associated with DHF grades II-IV ($p\leq0.2$). These parameters

Table 2
Baseline characteristics and laboratory findings among subjects classified by the revised 2009 WHO definitions.

Characteristics	Severe dengue		Non-severe dengue with warning signs		*p*-value
	n	Median (IQR[a])	*n*	Median (IQR[a])	
Baseline characteristics					
Age (years)	90	23.5 (20.0-33.3)	193	24.0 (20.0-34.5)	0.994
Gender: female, no. (%)	90	60 (66.7)	193	78 (40.4)	<0.001
Medical illnesses, no. (%)	90	23 (25.6)	193	39 (20.2)	0.390
Fever (days)	90	4.0 (3.0-5.0)	193	4.0 (3.0-5.0)	0.087
Headache, no. (%)	90	64 (71.1)	193	134 (69.4)	0.882
Vomiting, no. (%)	90	58 (64.4)	193	117 (60.6)	0.628
Myalgia or arthralgia, no. (%)	90	45 (50.0)	193	114 (59.1)	0.193
Abdominal pain, no. (%)	90	30 (33.3)	193	69 (35.8)	0.792
Rash, no. (%)	90	19 (21.1)	193	41 (21.2)	1.000
Retro-orbital pain, no. (%)	90	3 (3.3)	193	8 (4.1)	1.000
Physical examinations					
Temperature (°C)	90	38.0 (37.4-38.5)	193	37.8 (37.0-38.5)	0.197
Mean arterial pressure (mmHg)	90	80.2 (73.3-86.7)	193	83.3 (80.0-93.3)	<0.001
Petechiae, no. (%)	90	23 (25.6)	193	52 (26.9)	0.919
Laboratory findings					
Hemoglobin (g/dl)	90	13.5 (12.2-15.0)	193	14.6 (13.3-15.5)	<0.001
Hematocrit (%)	90	40.7 (36.8-44.4)	193	43.5 (40.0-45.9)	<0.001
White cell counts (x10^3/ l)	90	3.0 (2.0-4.2)	193	2.9 (2.1-4.2)	0.857
Lymphocytes (%)	90	27.1 (18.0-40.4)	193	31.0 (22.0-42.8)	0.201
Atypical lymphocytes (%)	90	2.5 (0-9.3)	192	5.0 (0-11.0)	0.087
Platelet count (x10^3/ l)	90	69.5 (40.0-108.3)	193	73.0 (48.0-96.0)	0.818
AST[b] (IU/l)	67	131.0 (53.0-339.0)	144	111.5 (64.5-166.8)	0.166
ALT[c] (IU/l)	67	75.0 (37.0-121.0)	145	72.0 (45.0-123.0)	0.940
Albumin (g/dl)	38	4.0 (3.8-4.4)	75	4.2 (3.9-4.4)	0.140
Serum creatinine (mg/dl)	57	0.8 (0.7-1.0)	119	0.8 (0.7-1.0)	0.838
Categorical data					
Hematocrit <2%, no (%)	90	72 (80.0)	193	122 (63.2)	0.007
Mean arterial pressure <80	90	38 (42.2)	193	40 (20.7)	<0.001

[a]IQR, interquartile range; [b]AST, aspartate aminotransferase; [c]ALT, alanine aminotransferase

were then further analyzed with stepwise multivariate logistic regression analysis using the backward selection method. A Hct >2% above the reference range [OR (95% CI), 3.235 (1.807-5.793), $p<0.001$] and an ALT >120 IU/l [1.896 (1.018-3.531), $p=0.044$] were factors independently associated with DHF grades II-IV.

Following the revised 2009 WHO definitions for severe clinical manifestations, univariate analysis showed female gender [OR (95% CI), 2.949 (1.746-4.980), $p<0.001$], a MAP <80 mmHg [2.795 (1.622-4.817), $p<0.001$], and a Hct <2% below the reference range [1.820 (1.069-3.099), $p=0.027$] were factors significantly asso-

Table 3
Univariate and multivariate analysis of clinical and laboratory parameters on among subjects classified by the 1997 and revised 2009 WHO definitions.

Characteristics	Univariate analysis		Multivariate analysis	
	OR[a] (95% CI[b])	*p*-value	OR[a] (95% CI[b])	*p*-value
DHF grades II-IV				
Hematocrit >2%	3.272 (1.985-5.393)	<0.001	3.235 (1.807-5.739)	<0.001
ALT[c] >120 IU/l	2.057 (1.128-3.753)	0.019	1.896 (1.018-3.531)	0.044
Severe dengue				
Female gender	2.949 (1.746-4.980)	<0.001	2.042 (1.143-3.648)	0.016
Mean arterial pressure < 80	2.795 (1.622-4.817)	<0.001	2.275 (1.302-3.975)	0.004
Hematocrit <2%	1.820 (1.069-3.099)	0.027	1.468 (0.838-2.572)	0.179

[a]OR, odds ratio; [b]CI, confidence intervals; [c]ALT, alanine aminotransferase

ciated with severe dengue ($p \leq 0.2$). These parameters were then further analyzed with stepwise multivariate logistic regression analysis using the backward selection method. Female gender [OR (95% CI), 2.042 (1.143-3.648), $p=0.016$] and a MAP <80 mmHg [2.275 (1.302-3.975), $p=0.004$] were factors independently associated with severe dengue.

DISCUSSION

In Thailand, the incidence of adult patients with dengue has been increasing since 1960 (Nimmannitya, 1987). The common complications of adult patients with dengue infection include bleeding, thrombocytopenia, hemoconcentration and increased liver enzymes, which is different from children (Wichmann *et al*, 2004; Kittigul *et al*, 2007). Previous studies of factors associated with severe dengue infection in adults are limited and the results vary widely due to differences in study design, outcomes and data analysis. Some studies have found Chinese ethnicity, female gender, history of medical illnesses, such as asthma, hypertension and diabetes, secondary dengue infection, clinical bleeding, lower lymphocyte counts, lower platelet counts, lower total protein levels, higher blood urea nitrogen and higher liver enzyme levels were factors associated with severe dengue infection in adults (Burke *et al*, 1988; González *et al*, 2005; Wichmann *et al*, 2007; Lee *et al*, 2008; Figueiredo *et al*, 2010).

In our study, 323 medical records of adult hospitalized patients with dengue infection were reviewed. DHF grades II-IV following 1997 WHO definitions was observed in 161 patients, of whom 99 (61.5%) had plasma leakage with hypotension or shock. Most of patients (72/90, 80%) with severe dengue following revised 2009 WHO definition had severe clinical bleeding during hospitalization. Therefore, plasma leakage and clinical bleeding were common complications of adult patients with dengue in this study, similar to the previous reports (Bandyopadhyay *et al*, 2006; Thomas *et al*, 2010). Clinical bleeding in patients with dengue infection can occur with or without plasma leakage (Bandyopadhyay *et al*, 2006; Thomas *et al*, 2010). Hemoconcentration,

lower platelet counts and higher liver enzymes were observed in patients with DHF grades II-IV, whereas female gender, lower MAP, lower Hb and lower Hct were observed in patients with severe dengue. Our findings are similar to a previous study showing that the presence of severe clinical bleeding in dengue patients can result in a decrease in Hct and Hb levels and a low MAP during defervescence (Lum *et al*, 2002). In contrast, the presence of hemoconcentration and increased liver enzymes in dengue patients may occur from plasma leakage, which leads to tissue edema and intravascular volume depletion (Sahaphong *et al*, 1980).

On multivariate analysis, the independent factors associated with DHF grades II-IV were a Hct >2% above the reference range and an ALT level >120 IU/l; female gender and a MAP <80 mmHg were factors independently associated with severe dengue. All the patients with severe clinical manifestations survived in our study. It is possible these patients received early management, close observation and prevention of hemorrhagic bleeding. Proton pump inhibitors were used in patients with gastrointestinal bleeding and those at high risk for gastrointestinal bleeding, such as those with thrombocytopenia and severe vomiting. During defervescence, blood pressure, pulse pressure and urine output were recorded every 2-4 hours while Hct and urine specific gravity were recorded every 6-8 hours in order to adjust the rate of intravenous fluid to keep the patient hemodynamically stable.

In conclusion, dengue patients with a Hct >2% above the reference range or with an ALT level >120 IU/l on admission were at increased risk of developing DHF grades II-IV, of which plasma leakage with hypotension and shock were the most common complications during hospitalization. Female gender or a MAP <80 mmHg on admission were also risk factors for severe dengue infection, of which severe bleeding was the most common complication during hospitalization. These findings can be used as indicators for severe dengue infection and may help clinicians to provide proper management, to reduce complications in dengue patients.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. We would like to thank all the nurses and staff at registry unit of the Hospital for Tropical Diseases, Bangkok, Thailand for collecting and retrieving the medical records. Special thanks are extended to Dr Brendan Arnold for reading and making helpful comments on the manuscript and Assoc Prof Pratap Singhasivanon, Dean of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand for his support with this manuscript.

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